


# Higher Preoperative Plasma Thrombin Potential in Patients Undergoing Surgery for Aortic Stenosis Compared to Surgery for Stable Coronary Artery Disease

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## Abstract

Aortic stenosis (AS) and coronary artery disease (CAD) influence the coagulation system, potentially affecting hemostasis during cardiac surgery. Our aim was to evaluate 2 preoperative global hemostasis assays, plasma thrombin potential and thromboelastometry, in patients with severe aortic valve stenosis compared to patients with CAD. A secondary aim was to test whether the assays were associated with postoperative bleeding. Calibrated automated thrombogram (CAT) in platelet-poor plasma and rotational thromboelastometry (ROTEM) in whole blood were analyzed in patients scheduled for elective surgery due to severe AS ( $n = 103$ ) and stable CAD ( $n = 68$ ). Patients with AS displayed higher plasma thrombin potential, both thrombin peak with median 252 nmol/L (interquartile range 187-319) and endogenous thrombin potential (ETP) with median 1552 nmol/L/min (interquartile range 1340-1838), when compared to patients with CAD where thrombin peak was median 174 nmol/L (interquartile range 147-229) and ETP median 1247 nmol/L/min (interquartile range 1034-1448; both  $P < .001$ ). Differences persisted after adjustment for age, gender, comorbidity, and antithrombotic treatment. Differences observed in thromboelastometry between the groups did not persist after adjustment for baseline characteristics. Bleeding amount showed no relationship with plasma thrombin potential but weakly to thromboelastometry ( $R^2 = .064$ ,  $P = .001$ ). Patients with AS exhibited preoperatively increased plasma thrombin potential compared to patients with CAD. Plasma thrombin potential was not predictive for postoperative bleeding in patients scheduled for elective surgery.

## Keywords

bleeding, hemostasis, in vitro diagnostic systems

## Introduction

Aortic valve stenosis (AS) and coronary artery disease (CAD) are acquired cardiac diseases that frequently coexist and share epidemiological risk factors and are commonly surgically treated.<sup>1,2</sup> Postoperative bleeding is a major cause of morbidity and mortality in cardiac surgery.<sup>3-5</sup> Aortic valve disease has been identified as a risk factor for excessive postoperative bleeding.<sup>6</sup>

The coagulopathy seen in patients with AS has not been fully explored. Increased thrombin generation (TG; evaluated in vivo), platelet activity, and hyperfibrinolysis have been reported.<sup>7-10</sup> The microparticle-induced TG in AS has recently been found to be related to the degree of coronary atherosclerosis rather than the aortic calcification.<sup>11,12</sup> It is established that the severity of coronary atherosclerosis in patients with stable CAD is associated with an increased TG both evaluated in vivo as thrombin-antithrombin complex and evaluated in vitro as

endogenous thrombin potential (ETP).<sup>11,13,14</sup> Others have described impaired von Willebrand factor (vWF) and reduced platelet aggregation in severe AS when compared to healthy individuals.<sup>9</sup> Patients with AS have a risk of gastrointestinal bleeding related to altered hemostasis, and the change in vWF factor multimer structure and vWF activity has been associated with increased postoperative drainage after surgery for AS.<sup>15,16</sup>

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Thromboelastometry is now the standard bedside test in situations where excessive bleeding complicates cardiac surgery, and it provides rapid data on the speed, firmness, and stability of clot formation. Treatment of bleeding guided by rotational thromboelastometry (ROTEM) reduces the need for transfusions in cardiac surgery.<sup>17</sup> Calibrated automated thrombogram (CAT) assesses the coagulation factors' entire capability to generate thrombin in plasma, in contrast to time-based clotting tests where only a few percentage of the total amount of potential thrombin is needed to start clotting.<sup>18</sup> The CAT assays are time consuming and still not used in the clinical bedside setting, but reports of association between preoperative TG measured with CAT and postoperative bleeding in coronary artery bypass grafting (CABG) patients have been published.<sup>19,20</sup>

Our aim was to evaluate preoperative plasma thrombin potential and clot formation in patients with severe AS compared to CAD planned for thoracic surgery. Secondary aims were to evaluate whether plasma thrombin potential and thromboelastography assessed preoperatively were associated with postoperative bleeding and to explore associations between the 2 assays.

## Materials and Methods

### Study Design and Patient Population

A prospective, observational, single-center study was designed. Patients planned for operation for isolated severe AS ( $n = 103$ ) and stable multivessel CAD ( $n = 68$ ) between November 2013 and March 2015 at Uppsala University hospital were included. Predefined exclusion criteria were age  $<18$  years, unstable angina or myocardial infarction within 6 months, previous sternotomy, and off-pump surgery. All patients with AS underwent preoperative coronary angiography without findings of coronary disease eligible for bypass grafting.

Preoperative patient baseline characteristics and operative and postoperative outcomes were prospectively recorded into a dedicated database. Postoperative bleeding was predefined as total chest tube output during the first 12 hours after sternal closure. The study was approved by the local ethics committee, and all participants gave written informed consent.

### Antithrombotic Treatment and Cardiopulmonary Bypass

According to local routine based on European guidelines,<sup>21</sup> clopidogrel was discontinued 5 days and warfarin 3 days before surgery, while acetylsalicylic acid treatment was not discontinued. Aprotinin and dextran infusions were not used throughout the study period. Patients preoperatively received 2 g of tranexamic acid before and 2 g after cardiopulmonary bypass (CPB).

Heparin and protamine (Leo Pharmaceutical Products BV, Weesp, the Netherlands) dosage was guided by repeat Hepcon (Medtronic, Minneapolis, Minnesota) measurements to reach activated clotting time (ACT)  $>480$  seconds and then reversed to baseline. Nonpulsatile roller pump CPB with membrane oxygenator, primed with 1600 to 1800 mL Ringer-Acetate and 10 000 E heparin was used. Cardioprotection was achieved

with repeated cold blood cardioplegia. Lowest temperature ranged between  $32^{\circ}\text{C}$  and  $36^{\circ}\text{C}$  during CPB. According to local routine, the hematocrit was maintained over 21% during CPB and 27% after CPB with transfusion of packed red blood cells if needed; however, the final decision for transfusion was up to the attending anesthesiologist. Patients on anticoagulant treatment with suspected residual effect of the drug were given 2 units of plasma at the end of surgery. No colloid fluids were administered during surgery. Cell salvage device was not used routinely but requested intraoperatively in 2 cases. Shed mediastinal blood was reintroduced into the CPB system.

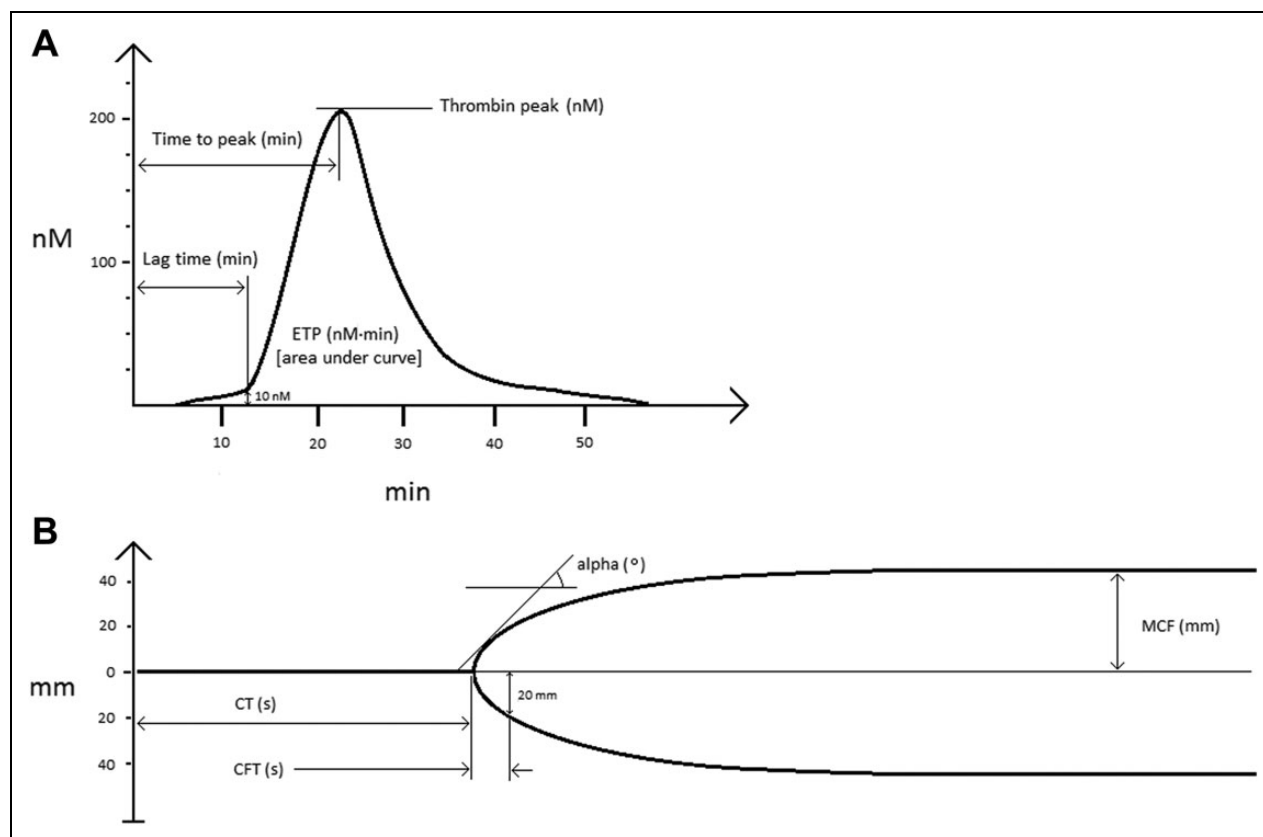
### Blood Samples

Samples for analysis of plasma thrombin potential by CAT were collected preoperatively from the cubital vein, without stasis, at arrival at the clinic and for thromboelastography by ROTEM before induction of anesthesia from the arterial line. Platelet-poor plasma for CAT analysis and fibrinogen concentration was prepared from 3.5-mL vacutainer tubes containing 3.2% sodium citrate (Greiner Bio-One GmbH, Kremsmünster, Austria), delivered to the laboratory within 2 hours, spun at 2000g for 20 minutes, and stored in 125- $\mu\text{L}$  aliquots at  $-80^{\circ}\text{C}$  until analysis. Fibrinogen plasma concentration was analyzed with Clauss method with STA Liquid Fib reagent (STAGO Diagnostica & Roche, Düsseldorf, Germany). The coefficient of variances for the method was 7%. Whole blood samples for ROTEM were collected in 3.5-mL vacutainer tubes containing 3.2% sodium citrate (Greiner Bio-One GmbH), maintained at  $37^{\circ}\text{C}$ , and analyzed within 30 minutes.

### Plasma Thrombin Potential

Plasma thrombin potential assay was performed using CAT (Thrombinoscope, Maastricht, the Netherlands) measured in a 96-well plate fluorometer (Fluoroskan Ascent; ThermoScientific, Waltham, Massachusetts). The general principles are described previously.<sup>22</sup> In this study, 80  $\mu\text{L}$  of plasma was mixed with 20  $\mu\text{L}$  of phospholipids (MP-reagent; Diagnostica Stago, Asnières, France). Samples spiked with 20  $\mu\text{L}$  of thrombin calibrator (Thrombinoscope, Maastricht, the Netherlands) were run in parallel with each cycle of test samples. The fluorometric measurements were performed after automated addition of 20  $\mu\text{L}$  FluCa-kit (417  $\mu\text{mol/L}$  fluorescent substrate Z-Gly-Gly-Arg-AMC and 16.7  $\text{nmol/L}$   $\text{CaCl}_2$ , final concentrations). No exogenous tissue factor was added in this assay.<sup>23</sup> The process was monitored for 60 minutes. Samples were run in duplicate, and the mean value was calculated.

The following values were registered: time to detection of thrombin (lag time) and to highest thrombin concentration (peak) in minutes, peak thrombin concentration (thrombin peak) in  $\text{nmol/L}$ , and ETP, defined as area under the curve, in  $\text{nmol/L/min}$  (Figure 1). The coefficient of variances was 15% and 10% for thrombin peak and ETP, respectively. Analyses without a significant curve were categorized as no result.



**Figure 1.** Exemplification of result curves of (A) plasma thrombin concentration in calibrated automated thrombogram (CAT) and (B) clot firmness in rotational thromboelastometry (ROTEM). Lag time indicates time from start until 10 nmol/L of thrombin is detected; time to peak, the time until maximal concentration is reached; peak, maximum concentration registered; ETP, endogenous thrombin potential—area under the curve; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness.

### Rotational Thromboelastometry

Rotational thromboelastometry (ROTEM delta; TEM International GmbH, Munich, Germany) analyses were conducted as specified by the manufacturer. The method is described previously.<sup>24</sup> Extrinsic thromboelastometry activated with tissue factor (EXTEM), intrinsic thromboelastometry activated with ellagic acid and phospholipid (INTEM), and fibrinogen thromboelastometry activated with tissue factor with addition of cytochalasin platelet inhibition (FIBTEM) were analyzed and run for 60 minutes.

The following values automatically calculated by the manufacturer's software were registered: time to first registered clotting (clotting time, CT) and time from first registered clotting to 20-mm clot firmness (clot formation time, CFT) in seconds, highest clot firmness registered (maximum clot firmness, MCF) in mm, and angle of initial curve ( $\alpha$ ) in degrees (Figure 1). Erroneous results, as indicated by the ROTEM device, were excluded.

### Statistical Analysis

Continuous data are presented as median (interquartile range) and categorical data as frequency and percentage (%). After testing bleeding amount, ROTEM and CAT variables for

normality and equality variance with Shapiro-Wilk and Levene test, nonparametric tests, were used. Comparisons between group means were done with independent sample Mann-Whitney *U* test for continuous variables and chi-square test for categorical variables. Correlations were calculated with Spearman correlation. Association with bleeding was tested with univariate linear regression using the natural logarithm of postoperative bleeding amount as dependent variable. A *P* value under .05 was considered significant. Wilcoxon nonparametric residual method was used to adjust results for interaction of differences in anticoagulant treatment as well as baseline variables with significant differences between the groups.<sup>25</sup> A power analysis was not performed due to the study's observational and explorative design.

Statistical analyses were performed using SPSS (IBM SPSS Statistics for Windows, version 22.0. IBM Corp, Armonk, New York, USA) and SAS version 9.4 (SAS Institute, Cary, North Carolina, USA).

## Results

### Patient Characteristics

The study group consisted of 171 patients, of which 103 underwent isolated aortic valve replacement due to AS and 68

**Table 1.** Patient Characteristics and Postoperative Course of Events.<sup>a</sup>

|  | Aortic Stenosis, n = 103 | Coronary Artery Disease, n = 68 | P Value |
|--|--------------------------|---------------------------------|---------|
| <b>Preoperative characteristics</b>                |                          |                                 |         |
| Age, years   | 70 (62-76)               | 68 (64-70)                      | .29     |
| Male gender  | 59 (57%)                 | 61 (90%)                        | <.001   |
| Diabetes with or without insulin                   | 17 (17%)                 | 22 (32%)                        | .016    |
| Hypertension                                       | 58 (56%)                 | 55 (81%)                        | .001    |
| Euroscore II                                       | 1.47 (0.98-2.02)         | 1.16 (0.83 -1.81)               | .15     |
| Left ventricular ejection fraction <50%            | 16 (16%)                 | 18 (26%)                        | .08     |
| Previous significant bleeding <sup>b</sup>         | 15 (15%)                 | 10 (15%)                        | .98     |
| Previous arterial or venous embolus <sup>c</sup>   | 18 (17%)                 | 7 (10%)                         | .20     |
| Atrial fibrillation                                | 10 (10%)                 | 3 (4%)                          | .20     |
| <b>Preoperative laboratory parameters</b>          |                          |                                 |         |
| Hemoglobin, g L <sup>-1</sup>                      | 136 (128-145)            | 143 (137-150)                   | <.001   |
| Platelets, × 10 <sup>9</sup> L <sup>-1</sup>       | 237 (184-237)            | 225 (193-265)                   | .24     |
| CRP, mg/L  | 1.9 (1.0-4.3)            | 1.5 (0.8-2.5)                   | .08     |
| APTT, s  | 36 (34-39)               | 35 (32-37)                      | .06     |
| INR  | 1.0 (1.0-1.1)            | 1.0 (1.0-1.1)                   | .32     |
| Fibrinogen, g L <sup>-1</sup>                      | 3.5 (3.1-4.0)            | 3.6 (3.0-4.1)                   | .76     |
| <b>Preoperative antithrombotic treatment</b>       |                          |                                 |         |
| Acetylsalicylic acid                               | 30 (29%)                 | 61 (90%)                        | <.001   |
| Clopidogrel  | 5 (5%)                   | 12 (18%)                        | .006    |
| Ticagrelol   | 0 (0%)                   | 1 (1%)                          | .22     |
| Warfarin   | 11 (11%)                 | 3 (4%)                          | .15     |
| Apixaban   | 1 (1%)                   | 2 (3%)                          | .34     |
| Rivaroxaban  | 1 (1%)                   | 0 (0%)                          | .42     |
| <b>Operative and postoperative characteristics</b> |                          |                                 |         |
| Aortic occlusion time, min                         | 72 (60-88)               | 52 (42-67)                      | <.001   |
| Intraoperatively bleeding, mL                      | 500 (400-800)            | 600 (400-900)                   | .22     |
| Postoperatively bleeding 0 to 12 hours, mL         | 290 (230-450)            | 410 (320-560)                   | .001    |
| Reexploration due to bleeding                      | 4 (4%)                   | 2 (3%)                          | .74     |
| 30-day total mortality                             | 0 (0%)                   | 1 (1%)                          | .22     |
| Erythrocytes (units) <sup>d</sup>                  | 1.3 ± 1.8                | 0.9 ± 1.2                       | .39     |
| Plasma, units <sup>d</sup>                         | 0.3 ± 0.8                | 0.0 ± 0.3                       | .03     |
| Platelets, units <sup>d</sup>                      | 0.4 ± 0.9                | 0.3 ± 0.7                       | .72     |

Abbreviations: APTT, activated partial thromboplastin time; CRP, C-reactive protein; INR, international normalized ratio.

<sup>a</sup>Data are median (interquartile range), mean ± standard deviation or number (%).

<sup>b</sup>Gastrointestinal, urothelial or cerebral.

<sup>c</sup>Including thromboembolic stroke, transitory ischemic attack, deep vein thrombosis and pulmonary embolus.

<sup>d</sup>Total units transfused until discharge.

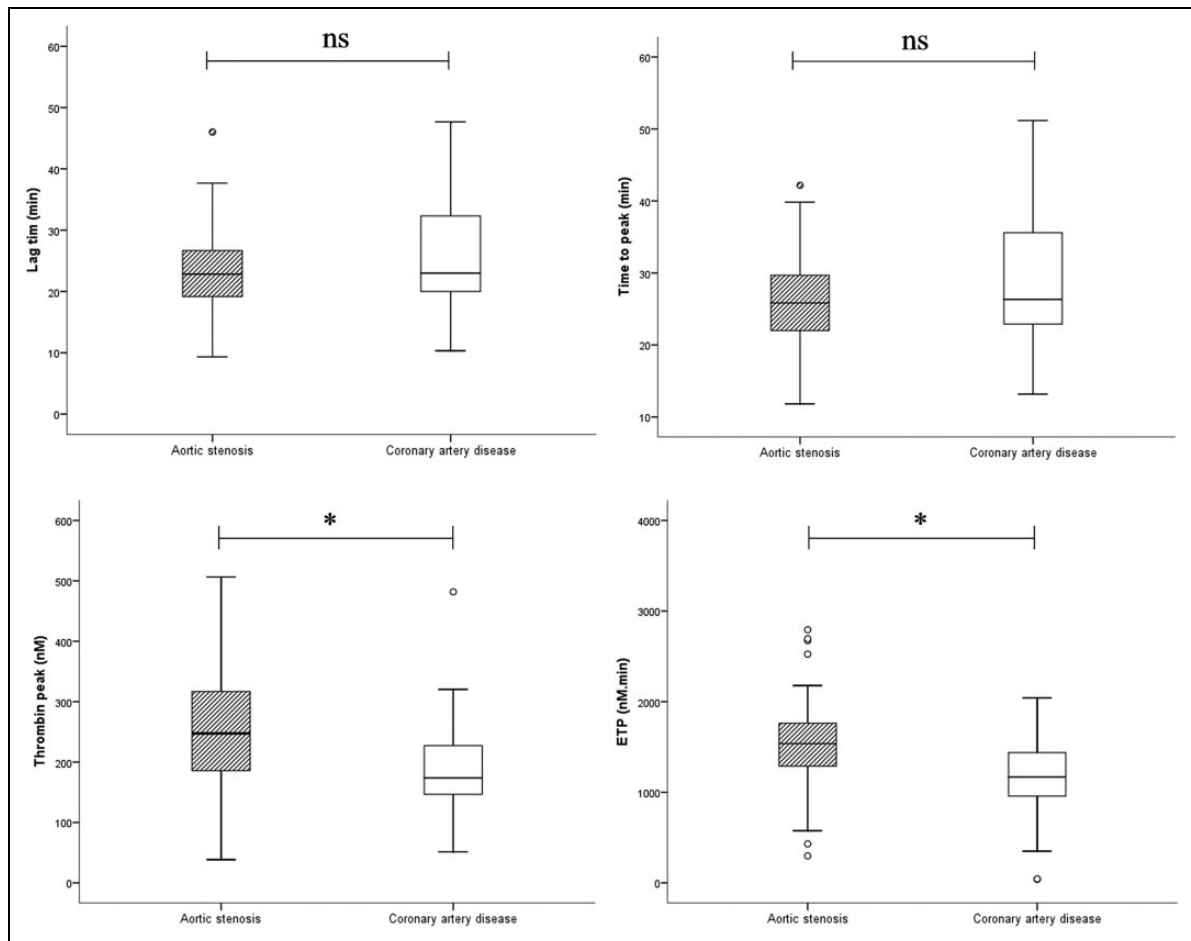
isolated coronary artery bypass grafting due to stable CAD. Demographic and clinical parameters are listed in Table 1. Age, Euroscore II, and history of bleeding or embolus showed no difference between the groups. Male sex, hypertension, and diabetes were less common and preoperative hemoglobin lower in the AS group. Aortic occlusion time was longer in the AS group, while total time on CPB did not differ. Intraoperative bleeding, reexploration due to bleeding, and transfusions did not differ between the groups. Postoperative median bleeding amount during the first 12 hours was lower among patients with AS, median 290 mL, than in patients with CAD, median 410 mL ( $P = .001$ ).

### Plasma Thrombin Potential

Plasma thrombin potential was higher in the AS group; thrombin peak median (interquartile range) was 252 nmol/L (187-319) and 174 nmol/L (147-229) in patients with AS and CAD,

respectively, and median ETP was 1552 nmol/L/min (1340-1838) and 1247 nmol/L/min (1034-1448), respectively ( $P < .001$ ; Figure 2, Table 2). No difference was found in the lag time and time to peak when comparing the groups. CAT curves classified as no results were found in 7.0% of patients. Thrombin peak and ETP persisted significantly higher in the AS compared to CAD group ( $P = .0019$  and  $P = .0008$ , respectively) after adjustment for the baseline characteristics age, gender, hypertension, diabetes, hemoglobin, antiplatelet therapy, and warfarin treatment.

Plasma thrombin potential was decreased in patients with AS with preoperative anticoagulation treatment ( $n = 13$ ) compared to those without preoperative anticoagulation treatment ( $n = 90$ ). The ETP was 886 nmol/L/min (503-1333) and 1566 nmol/L/min (1341-1840) ( $P < .001$ ) in patients with and without preoperative anticoagulation treatment, respectively, and thrombin peak was 151 nmol/L (90.9-207) and 260 nmol/L (197-330) nM ( $P = .001$ ),



**Figure 2.** Preoperative plasma thrombin potential in patients with aortic stenosis and coronary artery disease. Thrombin generation measured with calibrated automated thrombogram. Statistical significance was assessed by independent sample Mann-Whitney *U* test. ETP indicates endogenous thrombin potential. \*Statistically significant ( $P < .01$ ), ns = nonsignificant. °Outlier ( $>1.5 \times$  interquartile range from 25th to 75th percentile).

**Table 2.** Plasma Thrombin Potential Measured With CAT and Thromboelastometry Measured With ROTEM.<sup>a</sup>

|                       | Aortic Stenosis, n = 103 | Coronary Artery Disease, n = 68 | P Value |
|-----------------------|--------------------------|---------------------------------|---------|
| <b>CAT</b>            |                          |                                 |         |
| Lag time, minutes     | 22.7 (18.9-26.6)         | 23.0 (19.7-32.3)                | .19     |
| Time to peak, minutes | 25.8 (21.5-29.7)         | 26.3 (22.7-35.7)                | .13     |
| Thrombin peak, nM     | 252 (187-319)            | 174 (147-229)                   | <.001   |
| ETP, nmol/L/min       | 1552 (1340-1838)         | 1247 (1034-1448)                | <.001   |
| <b>INTEM</b>          |                          |                                 |         |
| CT, seconds           | 170 (161-179)            | 172 (157-182)                   | .84     |
| CFT, seconds          | 67 (56-80)               | 77 (61-91)                      | .009    |
| Alpha, °              | 77 (74-79)               | 75 (72-77)                      | .003    |
| MCF, mm               | 66 (62-68)               | 64 (60-67)                      | .13     |
| <b>EXTEM</b>          |                          |                                 |         |
| CT, seconds           | 54 (49-59)               | 54 (50-60)                      | .52     |
| CFT, seconds          | 89 (75-107)              | 97 (80-117)                     | .08     |
| Alpha, °              | 76 (73-80)               | 75 (70-78)                      | .06     |
| MCF, mm               | 64 (60-67)               | 65 (60-67)                      | .78     |
| <b>FIBTEM</b>         |                          |                                 |         |
| MCF, mm               | 16 (13-19)               | 15 (13-18)                      | .041    |

Abbreviations: CAT, calibrated automated thrombogram; ETP, endogenous thrombin potential; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness.

<sup>a</sup>Values are median (interquartile range).

respectively. Anticoagulant treatment was ceased at mean 4.9 (range 3-8 days) before surgery. Bleeding amount was comparable in patients with and without anticoagulant treatment, with median 300 mL [240-510] and 290 mL [230-443], respectively ( $P = .43$ ). All 4 cases of re-exploration for bleeding occurred in the group without anticoagulant treatment. Only patients with AS were analyzed due to low percentage of patients with CAD with anticoagulant treatment.

### Rotational Thromboelastometry

Patients with AS had a shorter median INTEM CFT ( $P = .009$ ) and higher median INTEM  $\alpha$  angle ( $P = .003$ ) when compared to patients with CAD, both indicating faster clot formation in patients with AS (Table 2). Erroneous results ranged from 2.9% to 5.3% for the different ROTEM variables. No significant differences remained in INTEM CFT ( $P = .18$ ) or  $\alpha$  ( $P = .12$ ) after adjustment for baseline characteristics. The EXTEM results did not differ significantly between the groups. The FIBTEM MCF tended to be higher in patients with AS ( $P = .041$ ), although median fibrinogen plasma concentration was similar in AS [3.5 (3.1-4.0) g/L] and CAD groups [3.6 (3.0-4.1) g/L;  $P = .76$ ].

Thromboelastometry CT was slower in patients with AS with anticoagulation treatment ( $n = 13$ ) compared to those without ( $n = 90$ ) in INTEM CT [180 seconds (169-196) vs 169 seconds (161-177),  $P = .03$ ] and EXTEM CT [60 seconds (55-71) vs 53 seconds (48-58),  $P = .001$ ], while all other thromboelastometry variables showed no statistically significant difference. Only patients with AS were analyzed regarding effects of anticoagulant treatment.

### Correlation Between Plasma Thrombin Potential and Thromboelastometry

The INTEM CT was the only variable with significant correlation to all CAT variables, and the highest correlation coefficients were seen to thrombin peak ( $r = -0.35$ ,  $P = <.001$ ) and time to peak ( $r = 0.31$ ,  $P < .001$ ; Table 3).

### Association Between Assays and Postoperative Bleeding

In simple linear regression, the preoperative plasma thrombin potential variables showed no association with log postoperative bleeding in the total study cohort. Preoperative thromboelastometry rendered weak statistically significant association with bleeding amount in MCF in INTEM, EXTEM, and FIBTEM, of which EXTEM MCF had the highest  $R^2$  value to logarithmic postoperative bleeding. Thromboelastometry results merely explained 2.8% to 6.4% of variation in bleeding (Table 4).

### Discussion

In the present study, we show that patients with severe AS have an increased plasma thrombin potential compared to patients with stable CAD. In patients who were treated with

**Table 3.** Correlations Between Plasma Thrombin Potential Measured With Calibrated Automated Thrombogram and Thromboelastography.

|               | Lag Time |         | Time to Peak |         | Peak |         | ETP  |         |
|---------------|----------|---------|--------------|---------|------|---------|------|---------|
|               | r        | P Value | r            | P Value | r    | P Value | r    | P Value |
| <b>INTEM</b>  |          |         |              |         |      |         |      |         |
| CT            | .28      | <.01    | .31          | <.01    | -.35 | <.01    | -.22 | <.01    |
| CFT           | .02      | .80     | .05          | .58     | -.15 | .06     | -.19 | .03     |
| $\alpha$      | -.07     | .41     | -.09         | .26     | .18  | .03     | .23  | <.01    |
| MCF           | .09      | .30     | .07          | .42     | .08  | .33     | .13  | .15     |
| <b>EXTEM</b>  |          |         |              |         |      |         |      |         |
| CT            | .22      | <.01    | .20          | .01     | -.10 | .22     | -.09 | .30     |
| CFT           | -.07     | .37     | -.05         | .54     | -.14 | .08     | -.24 | <.01    |
| $\alpha$      | .00      | .98     | .00          | .96     | .06  | .49     | .15  | .09     |
| MCF           | .14      | .09     | .13          | .12     | .02  | .79     | .12  | .17     |
| <b>FIBTEM</b> |          |         |              |         |      |         |      |         |
| MCF           | .11      | .19     | .10          | .23     | -.01 | .88     | .07  | .41     |

Abbreviations: r, Spearman coefficient; ETP, endogenous thrombin potential; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness.

**Table 4.** Univariate Linear Regression for Dependent Variable Postoperative Log Bleeding 0 to 12 hours (mL).

|               | $\beta$ | $R^2$ | B      | SE (B) | P Value |
|---------------|---------|-------|--------|--------|---------|
| <b>CAT</b>    |         |       |        |        |         |
| Lag time      | .089    | 0.008 | 0.007  | .006   | 0.26    |
| Time to peak  | .079    | 0.006 | 0.006  | .006   | 0.32    |
| Thrombin peak | -.130   | 0.017 | -0.001 | .000   | 0.10    |
| ETP           | -.119   | 0.008 | 0.000  | .000   | 0.14    |
| <b>INTEM</b>  |         |       |        |        |         |
| CT            | .02     | 0.00  | 0.001  | .002   | 0.80    |
| CFT           | .083    | 0.007 | 0.002  | .002   | 0.29    |
| Alpha         | -.078   | 0.006 | -0.011 | .011   | 0.32    |
| MCF           | -.168   | 0.028 | -0.015 | .007   | 0.031   |
| <b>EXTEM</b>  |         |       |        |        |         |
| CT            | -.051   | 0.003 | -0.003 | .005   | 0.52    |
| CFT           | .135    | 0.018 | 0.002  | .001   | 0.09    |
| Alpha         | -.133   | 0.018 | -0.012 | .007   | 0.09    |
| MCF           | -.255   | 0.064 | -0.022 | .007   | 0.001   |
| <b>FIBTEM</b> |         |       |        |        |         |
| MCF           | -.246   | 0.060 | -0.033 | .010   | 0.002   |
| Fibrinogen    | -.151   | 0.023 | -0.114 | .058   | 0.051   |

Abbreviations:  $\beta$ , standardized regression coefficient; B, unstandardized regression coefficient;  $R^2$ , coefficient of determination; SE (B), standard error of unstandardized regression coefficient; CAT, calibrated automated thrombogram; ETP, endogenous thrombin potential; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness.

anticoagulant, which was more common among patients with AS, thrombin peak and ETP were decreased although treatment was stopped following clinical guidelines. Analyzed preoperatively, plasma thrombin potential did not prove to be useful for assessing risk of postoperative bleeding in elective surgery while thromboelastometry's association with bleeding was weak.

Both patients with AS and CAD have been described to have altered plasma thrombin potential in vivo. Natroska and

colleagues showed that patients with AS having deficiency in vWF have increased prothrombin fragment 1+2 and thrombin-antithrombin complexes compared to healthy individuals.<sup>9</sup> Plasma thrombin potential has previously been shown to be associated with coronary calcification in patients with AS, when induced by microparticles, and it has been described that patients with CAD undergoing CABG have higher concentrations of prothrombin fragment 1+2.<sup>12,26</sup> In contrast, in this study comparing a larger group of patients with severe AS and CAD, the increased plasma thrombin potential, evaluated as thrombin peak and ETP, was found in patients with AS without significant coronary stenosis. The mechanism behind these findings must be further investigated, but the increased amount of microparticles found in severe AS might express tissue factor on their surfaces prone to induce TG to a higher degree than in stable CAD.<sup>27,28</sup> The method used for evaluation of plasma thrombin potential without adding tissue factor has previously been described as sensitive for endogenous tissue factor but could also involve activation through the intrinsic pathway.<sup>23</sup> The high frequency of impairment of vWF found in AS leading to an altered plasma coagulation and platelet activity could also contribute to the high plasma thrombin potential as a mechanism to preserve the global hemostatic potential.<sup>9,15</sup>

Anticoagulant treatment before surgery in patients with AS reduced plasma thrombin potential and increased thromboelastometry CT. It has previously been described that ongoing warfarin treatment decreases the ETP in a concentration-dependent manner as would be expected due to decreased vitamin K-dependent coagulation factors.<sup>29</sup> Although warfarin was discontinued preoperatively, the persistent effect on plasma thrombin potential indicated that CAT is sensitive to minor reductions in coagulation factors.

Our results identify the initiation phase of INTEM CT, which measures time from activation with ellagic acid and phospholipids to start of solid clot formation, to be the thromboelastometry parameter with the highest correlation to plasma thrombin potential. The CAT was activated with phospholipid surface without any added tissue factor, similar to the activation reactant used in INTEM, which could accentuate a covariance between the tests.

A previous study by Bosch and colleagues of 30 CABG patients showed preoperative TG in both platelet-rich and platelet-poor plasma to be predictive of postoperative bleeding, with significantly different levels of thrombin peak and ETP in groups based on median bleeding volume.<sup>19</sup> The present study, including a substantially larger group of patients, does not point toward any association between preoperative plasma thrombin potential and postoperative bleeding amount. However, the results are not directly comparable as Bosch and colleagues used recombinant tissue factor for activation in the TG analysis, drew blood samples at the beginning of surgery before heparin administration, and as median bleeding amount was more than twice as high and counted during the first 20 postoperative hours in contrast to 12 hours in our study. It is possible that association between preoperative TG and bleeding amount only appears after bleeding amounts larger than what

occurred in our series of single procedure elective cases. The patients in the present study had severe AS, and after intervention of the aortic valve, an immediate restoration of the function of vWF has been reported, which leads to a change the hemostatic potential, which might have contributed to the lack of association with postoperative bleeding.<sup>30,31</sup>

Most studies on thromboelastometry's prediction of postoperative bleeding amount have focused on tests performed during or after surgery. In a study by Reinhöfer and colleagues including thromboelastometry examined before start of surgery, a preoperative FIBTEM MCF  $\leq 8$  mm was found to be the thromboelastometry variable with the highest predictive value for excessive postoperative bleeding.<sup>32</sup> The present study also identified FIBTEM MCF, together with EXTEM MCF, to have the highest association with bleeding amount. The association on group level was too low to be likely to be of any clinical use for individual patients.

Preoperative thromboelastometry but not TG assay correlated with postoperative bleeding amount, suggesting that clot firmness but not thrombin potential could be predictive of bleeding in elective AVR and CABG surgery. The cause of this discrepancy between the 2 global coagulation assays is not clear. Clot firmness measured with ROTEM is thrombin dependent, as thrombin cleaves fibrinogen into fibrin and activates cross-linkage with factor XIII. However, clot firmness is also dependent on other factors including function and availability of fibrinogen, factor XIII, platelets, erythrocytes, and plasmin-mediated thrombolysis. FIBTEM, with cytochalasin inactivated platelets, was also associated with postoperative bleeding. Therefore, it may be hypothesized that fibrinogen availability becomes a limiting factor for coagulation during bleeding sooner than a reduced TG potential in the studied patient groups.

Valve surgery and aortic valve disease have been suggested to be associated with an increased risk of postoperative bleeding.<sup>6</sup> Studies directly comparing bleeding risk in surgery for AS and CAD are scarce, but bleeding appears to be a larger problem in the AS group, as published data show a higher risk for re-exploration of bleeding after AS than CAD surgery.<sup>33-34</sup> The finding of a higher TG activity in patients with AS therefore does not seem to be accompanied by a more effective coagulation, and further studies evaluating the balance of primary hemostasis, coagulation, and fibrinolysis should be performed.

### Study Limitations

This study is limited by its single institution design and limited number of included patients. The protocol for CAT used in the present study did not include addition of tissue factor to induce TG which might contribute to a lower formation of thrombin. Corn trypsin inhibitor was not added in the protocol, and therefore, the intrinsic pathway could influence the initiation of coagulation evaluated by CAT. These factors might aggravate the comparison to previous studies, both for TG levels in the studied patient groups and when using the method to predict postoperative bleeding. Bleeding amount was generally low in

the study population of single procedure elective cases, and thus, any associations with the studied coagulation assays might only become evident in surgical procedures with higher risks of bleeding.

## Conclusion

Preoperative plasma thrombin potential is increased in patients with isolated AS when compared to in patients with stable CAD, while thromboelastometry does not differ between these patient groups. Evaluated preoperatively, bleeding was not associated with plasma thrombin potential and only to a lesser degree with thromboelastometry in patients undergoing elective AS and CAD surgery.


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